Markers of Altered Metabolism in Osteoarthritis

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For this discussion, I would like to consider biomarkers in the context of surrogate outcome measures for osteoarthritis (OA), and to make some broader comments regarding the use of outcome measures in OA, in general.

For the patient, obviously, OA presents a problem with pain and loss of function. We measure that in our studies with a variety of patient-relevant outcome instruments. These provide outcome measures that should form the core of any attempt to monitor the progression of OA or the effects of a therapeutic intervention. These are the "gold standard" outcomes.

Figure 1 shows the knee radiograph of a patient at 45 years of age, obtained because he had fairly persuasive symptoms of OA. The radiograph at that time was normal. The figure also shows a radiograph of the same patient's knee taken 13 years later, when his symptoms had become more severe and were now accompanied by severe structural changes of OA in the medial tibiofemoral compartment. This highlights one of the problems of using the radiograph to diagnose and monitor the progression of OA: it is insensitive to change. It takes a long time for things to happen in OA; many things may happen before we see anything on radiographic film. Indeed, if we look at 10 patients with severe medial compartment disease, only 4 will have symptoms consistent with what we call OA. The other 6 will be largely asymptomatic.

When treatment of OA pain is inadequate and the pain is too severe and function has been lost, we replace the joint. We would prefer, however, to detect cartilage loss or loss of joint function before it is too severe, in order to be able to prevent further loss. Possibly, at some point in the future we would also like to be able to facilitate regrowth of the lost cartilage. With those goals in mind, it is apparent that we have a problem in using some of the outcome measures we employ today to monitor OA.

In studies of potential disease-modifying OA drugs (DMOAD) and in population-based studies of the incidence of OA on a broader base, we need to select appropriate subjects (Figure 2). Whom should we select? Should we take a cross-section of the population or select only those with symptomatic OA? Should we select only those with radiographic changes? Or those with a combination of symptoms and radiographic changes, i.e., those with classic symptomatic and radiographic OA? Or, perhaps, we should select a subgroup of subjects at high risk for OA, such as those who are obese or have had previous joint injury. There are arguments for — and against — selecting any of these particular subpopulations, depending on the particular question we wish to ask. The problem we face is that we don't know the rate of transition from one group into another over time; the rate of progression of OA from preclinical to clinical disease, and within the spectrum of severity of the clinical disease, is highly variable. Progression rates vary markedly among individuals. This is a problem we face whether we use clinical, structural, or biological outcome measures.

In the remainder of my discussion I will deal with biological, or "process," markers, such as proteases or fragments of cartilage matrix macromolecules which, it has been suggested, may be useful outcome measures. What do we mean in this context by the term, "marker"? In general, markers are measured and evaluated as indicators of biologic or pathologic processes or indicators of the response to an intervention. In contrast, a clinical endpoint is a characteristic or variable that measures how a patient feels, functions, or survives. Those outcomes are at the core of anything we do as an intervention, whether within the context of a clinical trial or otherwise. That is the gold standard and what we fundamentally seek to evaluate. However, at times we attempt to construct a surrogate endpoint, i.e., a measure or marker that substitutes for a clinical endpoint, in an attempt to decrease the number of patients required for a clinical trial, or to shorten the duration of the trial. A marker, in itself, is not a surrogate endpoint. It becomes one only once we have validated it against the clinical endpoint — and therein lies the difficulty: How well do surrogate endpoints and measures reflect patient preference and quality of life? How do we evaluate beneficial effects of therapy that occur by pathways that are not really recognized by our potential surrogate measure? And what about adverse effects, which are not always accounted for by the surrogate endpoints we measure, but which may negate the apparent benefit of treatment?

Graphically, the above issues may be represented as a state of wellness and a state of disease and an intervention that mitigates the progression or conversion of one to the other. The best-case scenario is one in which the intervention influences the level of our biomarker by acting on a metabolic process, and in which a direct connection exists

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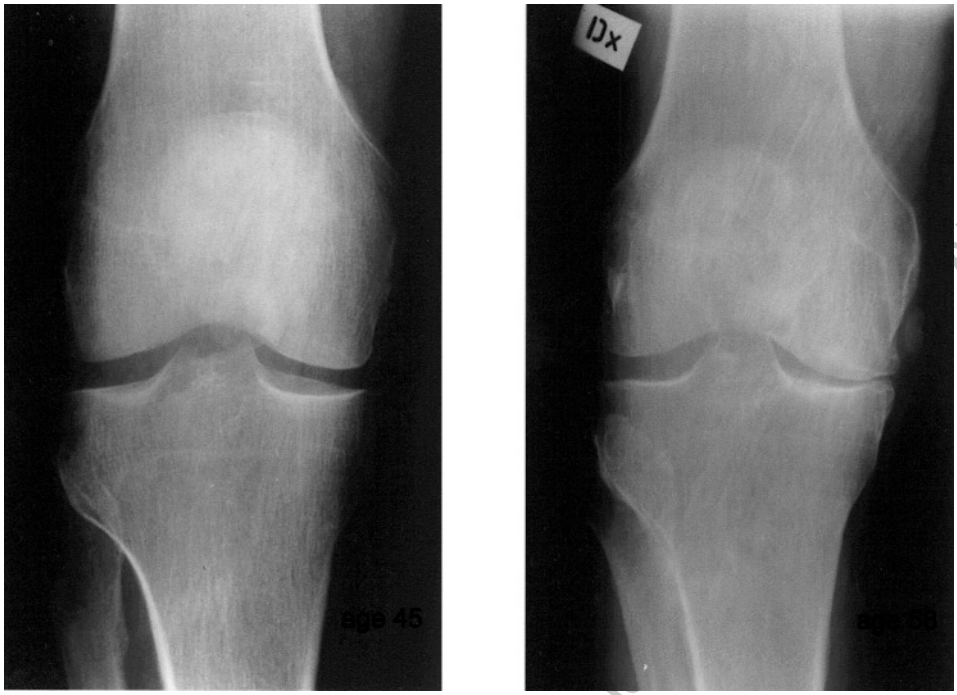

Figure 1. Standing AP radiographs of a patient who developed knee OA. The patient presented with joint pain and stiffness at 45 years of age, at which time the radiograph was normal (left panel). Symptoms gradually became more severe over the next 13 years. A radiograph then showed severe medial compartment OA with loss of joint space, subchondral sclerosis, and osteophytosis (right). In most patients with OA, the disease evolves slowly. The radiograph is relatively insensitive to change in OA.

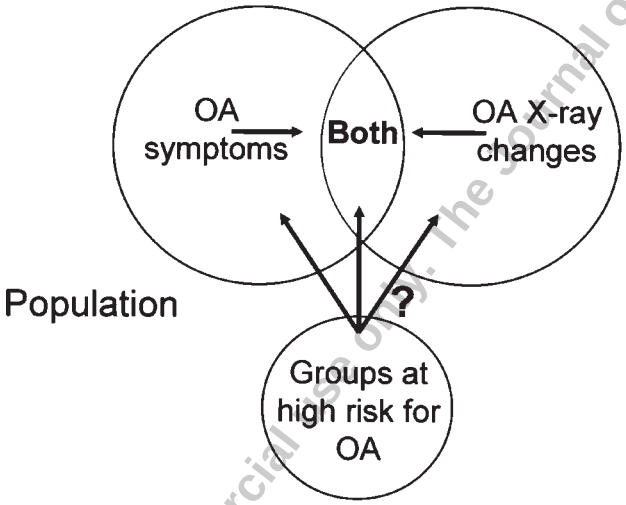

Figure 2. Possibilities for the selection of cohorts for OA clinical trials. Arrows depict the transition of an individual from one subgroup to another. In general, the transition rates are not well known.

between the change in the biomarker level and the disease state (Figure 3)[1]. In this situation it is possible that the level of the biomarker will correlate with the disease outcome, and thereby reflect a clinical endpoint. A more likely scenario is one in which the intervention affects endpoint and biomarker independently and where a proportion of the effect of the intervention is captured by the biomarker. If we can achieve that, we have done very well. However, we have not yet reached that point. At times, therefore, we are tempted to substitute irrelevant data that are easy to measure for relevant data that are more difficult to measure.

Figure 4 depicts a simplified view of the development of OA: against a genetically determined background of variable susceptibility and reactivity to damage, biomechanical or other insults (e.g., infection, muscle weakness) initiate signaling by cells within the joint and at other sites, leading to cartilage degradation and joint destruction. Although I'm going to focus on articular cartilage, it must be stated that OA is not simply a cartilage disease, but affects all tissues of the joint.

In OA a number of proteases within the cartilage attack the molecular network that is essential for the function and integrity of the articular cartilage. These proteases are generated both by the synovial membrane and by the chondrocytes, and result in degradation of the cartilage matrix and the generation of molecular fragments that are subsequently released from the cartilage into the synovial fluid (SF). From the joint fluid, these fragments — products of joint metabolism — are transported into the circulation, from which some are cleared by the kidney and appear in the urine[2]. This provides a basis for the discussion of molecular

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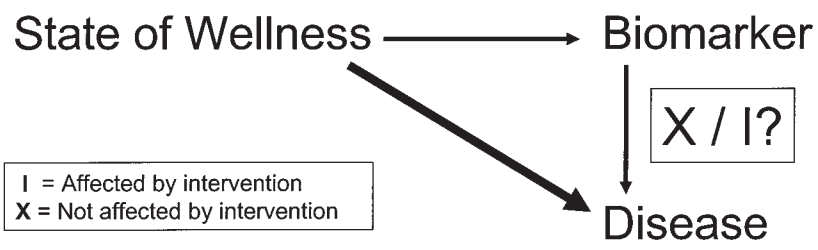

Figure 3. A biomarker may, at least in part, reflect the activity of a metabolic process related to the pathobiology or pathophysiology of OA. Theoretically, a therapeutic intervention that affected that process would affect the level of the biomarker, and modification of the metabolic activity would affect the patient's level of health or disease, defining a surrogate marker. In most cases, however, the relationship is imperfect or even lacking. Adapted from De Gruttola, et al. Control Clin Trials 2001;22:485-502.

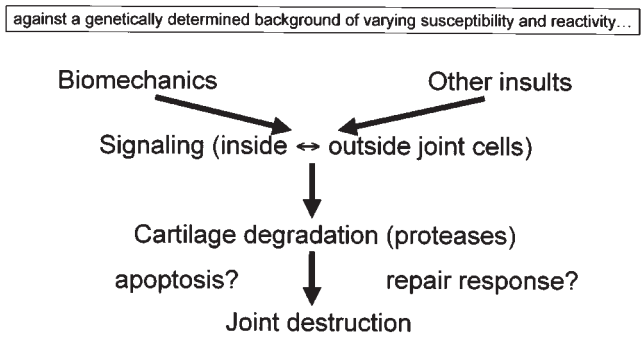

Figure 4. The development of OA. Endogenous (genetic) and exogenous (environmental) factors interact to initiate and drive the OA disease process. The variables depicted here have effects on joint tissues whose susceptibility to OA and reactivity to various stressors (e.g., ligament instability, varus-valgus malalignment, muscle weakness) is variable.

markers, i.e., biomarkers in OA, rheumatoid arthritis (RA), and various other joint disorders.

This scheme seems straightforward and simple, but it is far from that. As illustrated in Figure 5, we assume that matrix molecules that exit the joint cartilage appear in the SF, from which they are transported into the general circulation and eventually, at least partly, into the urine. That is an oversimplification, however, because the molecules may be actively metabolized in the liver or kidney. In addition, interchange occurs between the plasma and the interstitial fluid, and joints other than the index joint, as well as nonarticular sources, may contribute to the serum and urine levels of the marker of interest. Thus, the kinetics, i.e., the dynamics of movement and metabolism of the individual molecular fragments, are complex, making measurements of marker levels difficult to interpret[3]. Despite these problems, we are making progress and hope that we will eventually be

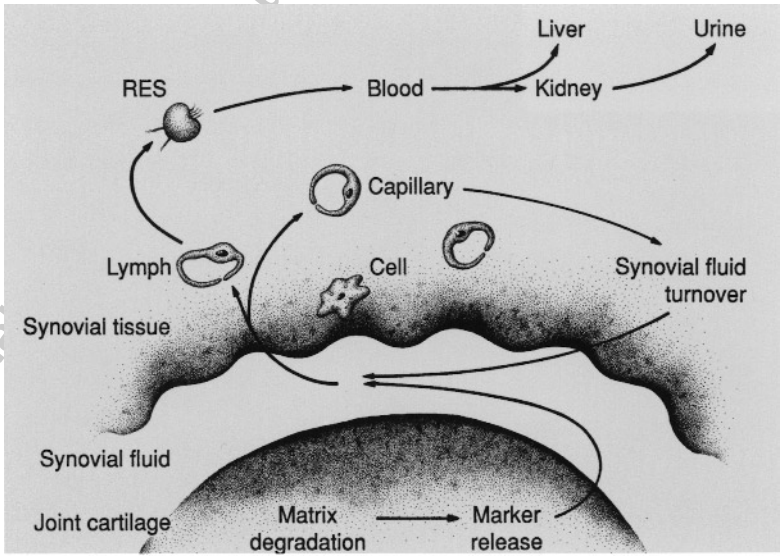

Figure 5. Biomarkers in body fluids. After their release from the articular cartilage, cartilage matrix molecules, or fragments thereof, travel through synovial fluid, plasma, and urine, in which their concentration may be quantified. Often, metabolic products generated in the joint are further processed in the more distal compartments. With permission, from Lohmander LS. Acta Orthop Scand 1991;62:623-32.

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able to use biomarkers to identify individuals with OA who are at risk for progression. In the future we would like to be able to use these measurements to monitor the effects of DMOAD therapy, particularly in the proof-of-concept stage of drug development, where a large problem exists today in interpreting outcomes, relative to disease modification. We may also use markers to explore mechanisms of disease and the dynamics of tissue changes within the OA joint.

What is the evidence that biomarkers can be used to predict the progression of OA? Some investigators have suggested that the serum hyaluronan (HA) concentration may relate to the size and numbers of OA joints involved, the rate of joint space narrowing, and to radiographic progression; that increased levels of C-reactive protein are associated with progression of radiographic knee OA; and that an increased serum concentration of cartilage oligomatrix protein (COMP) is associated with radiographic progression of knee and hip OA. Other reports have suggested the utility of other markers. This suggests the hypothesis that all of the markers mentioned above, and some I have not mentioned, may be markers of synovitis in patients with OA, i.e., they may all have some relationship to low-grade inflammation in the OA joint. Whether that is true for all patients with OA or only for a subset has yet to be determined. This is generally where things now stand with respect to the utility of markers for predicting progression of OA.

Can biomarkers serve as surrogate outcome measures in randomized clinical trials of DMOAD? The answer to that will not be known until we have identified an efficacious disease-modifying agent. However, related to this issue is the variability of the marker concentration within and between patients. This is relevant to the determination of how many patients are needed to detect a difference between the active treatment group and placebo group in a randomized clinical trial.

Figure 6 depicts data that were gathered in the context of a clinical trial, in which we obtained samples of SF, serum,

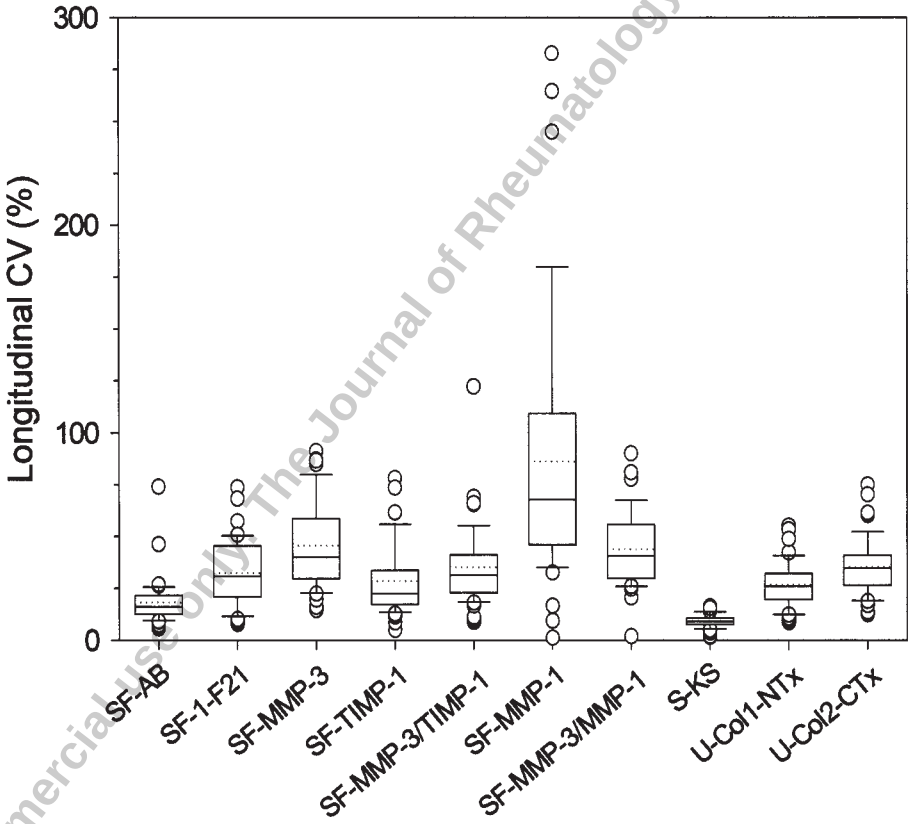

Figure 6. Within-patient variability in the concentration of 7 synovial fluid markers (SF-), 1 serum marker (S-), and 2 urinary markers (U-) in samples obtained from subjects on 8 occasions over a 12 month period. Results are expressed as the longitudinal coefficient of variation (CV) (%) (see text). CV of different markers may vary markedly, even within the same body fluid compartment. SF-AB: SF aggrecan fragments assayed by Alcian blue precipitation; SF-1-F21: SF aggrecan fragments determined by immunoassay; SF-MMP-3: stromelysin-1 protein determined by immunoassay; SF-TIMP-1: tissue inhibitor of metalloproteinases-1 determined by immunoassay; SF-MMP-1: collagenase-1 determined by immunoassay; SF-MMP-3/MMP-1: molar ratios of MMP-3 and MMP-1; S-KS: keratan sulfate determined by immunoassay; Col1-NTx: type I collagen N-telopeptide crosslink determined by immunoassay; Col2-NTx: type II collagen C-telopeptide crosslink determined by immunoassay. Modified from Lohmander, *et al*. Osteoarthritis Cartilage 1998;6:351-61.

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and urine from 51 patients on 8 occasions over the course of one year[4,5]. Because this trial failed to detect a difference in outcome between the drug that was being tested and placebo, data from subjects in both treatment groups have been combined. Notably, even among samples obtained from the same compartment (e.g., SF), the coefficients of variation of the different markers vary markedly within the same patient. This phenomenon is not assay-dependent, because the assay characteristics of each of these markers were more or less similar. Rather, the variability is biologically inherent among individual markers. SF levels of stromelysin protein suggest that if a decrease in the level of marker representing about one-half of one standard deviation is regarded as a relevant change, 30 patients per treatment arm would be sufficient to provide 80% power for detection of a difference between an active treatment and placebo. Thus, within-patient variability for this marker is fairly low.

Are biomarkers responsive to change? Analysis of samples from the clinical trial mentioned above was helpful in addressing this question, insofar as one of the patients developed septic arthritis of the knee during the course of the trial. The levels of aggrecan fragment in SF from that patient during the course of his joint infection are depicted in Figure 7 against the background of the other 51 patients in that cohort[5,6]. The changes were dramatic: during the acute joint infection the SF concentration rose some 35-fold. Following treatment of the infection, the sharp increase in the SF concentration of aggrecan fragments fell promptly back to the background level. Although the clinical event was dramatic, the data suggest the levels are, indeed, responsive to change.

Can biomarkers predict the response to a specific treatment? Over the past few years we have followed a number of patients longitudinally after a knee injury and have obtained serial samples from them. Figure 8 depicts cross-sectional analyses of matrix metalloproteinase-3 (MMP-3, stromelysin), measured at various intervals after injury, from SF in the same subjects[7]. The horizontal bar in the figure is the reference level for concentrations in SF from knees of healthy individuals. Dramatic increases occur after the injury, with the highest levels seen soon after the injury.

Similarly, measurement of SF collagenase protein levels (MMP-1) showed very high levels soon after injury, which then decreased over time but remained high for a very long period. Collagenase activity (rather than the concentration of enzyme protein) also rose markedly after the injury and the increase was sustained for as long as 100 weeks.

We have also examined levels of aggrecan degradation fragments, i.e., products of protease activity in SF. Figure 9 illustrates 2 aggrecan degradation pathways[8]. The major pathway *in vivo* is mediated by aggrecanases. Fragments of aggrecan generated by the action of aggrecanase can be detected in SF. We can measure the concentration of the

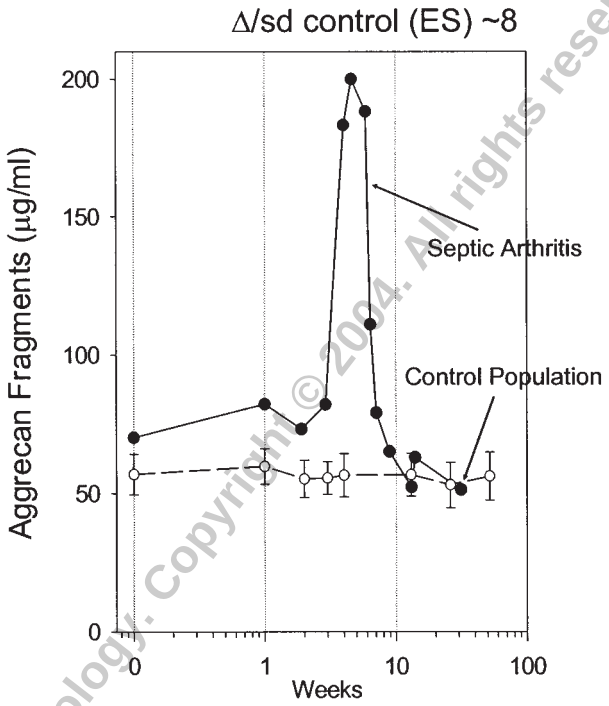

Figure 7. A. Concentration of aggrecan fragments in samples of SF obtained serially over a period of more than a year from a patient with OA. During this period the patient developed an acute joint infection, which resulted in an acute increase in the SF concentration of the marker, which fell promptly back to the baseline level after successful treatment of the infection. The mean level of the marker over time in samples from 51 reference subjects sampled in the same study is shown for comparison. Vertical bars on the plot for the control population depict 95% confidence intervals. The effect size (ES) is shown, calculated as the difference between the baseline and peak values for the study patient (A) divided by the standard deviation (SD) for the reference group. Modified from Lohmander, *et al*. Arthritis Rheum 2003;48:3130-9; and Christensson, *et al*. Acta Orthop Scand 1993;64:695-8.

enzyme protein, the level of protease activity, and the products of this activity, i.e., aggrecan fragments released from the cartilage into the SF. Again, in the SF samples discussed above, the highest concentrations occurred soon after the injury. However, this degradation does not affect all proteoglycan species similarly. As shown in Figure 10, which depicts some work we did in collaboration with Robin Poole, the ratio of the level of the 846 epitope to the level of total aggrecan changes over time after knee injury, suggesting that in this state of acute degradation of the matrix we also had catabolism of newly synthesized proteoglycan molecules and, possibly, a concomitant increase in the synthesis of new matrix proteoglycans[9].

We have also demonstrated increases in the SF concentration of COMP fragments in our cross-sectional studies of SF samples obtained at various intervals after knee injury, and have obtained immunochemical evidence of the degradation of Type II collagen, the framework of hyaline cartilage. Many types of collagen fragments are generated in the process of cartilage degradation after joint injury and can be

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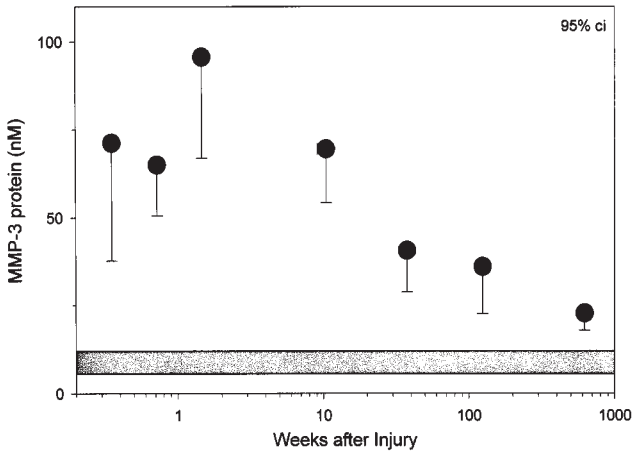

Figure 8. Cross-sectional analysis of the concentration of MMP-3 protein in SF from patients with joint injury, in relation to the time after injury, in weeks. Vertical bars show 95% confidence intervals. Horizontal band near the bottom depicts the range for the concentration of MMP-3 protein in SF from knees of healthy subjects. A dramatic increase in the concentration of the marker occurred after the injury, with the highest levels noted shortly after the injury. Modified from Lohmander LS, *et al*. J Rheumatol 1993;20:1362-8.

detected by various assays; David Eyre, Robin Poole, and others have developed assays that detect neoepitopes generated by enzymatic degradation of collagen. C-telopeptide region fragments of type II collagen are generated by a combination of MMP activity against the site indicated in Figure 11 and against another site further along the triple helix. The result is the release of a fragment containing the neoepitope attached to a crosslink, which is metabolically resistant to further breakdown[5,10].

With an assay developed by David Eyre that reflects the degradation of crosslinked Type II collagen we can show a high rate of release of fragments of this molecule into joint fluid soon after knee injury (Figure 12). Levels of the neoepitope in SF are as high after acute meniscus damage as after a tear of the anterior cruciate ligament (ACL). It is interesting to note, therefore, that patients are as likely to develop OA from a meniscus tear as from an ACL tear[11].

For a number of years we have been aware that very soon after joint injury a large quantity of proteoglycans and other cartilage products are released into the SF, from which they find their way into the circulation. Although we have argued that the collagen network does not really fall apart until much later, the above collagen data tell us this hypothesis is not correct; on the contrary, they indicate that the type II collagen network — the heart and core of the articular cartilage matrix — begins to fall apart very soon after injury.

After joint injury, surgeons tend to intervene late, with meniscectomy and ligament repair, and in ways that do not really affect the risk of these patients with respect to development of OA[11]. Nothing is done, however, to help the injured joint in the early phases, i.e., within a few weeks after injury. It would be very interesting to know whether intervention with a pharmacologic agent (DMOAD) in these early stages might affect the downstream development of OA. If the cartilage collagen network is damaged as a result

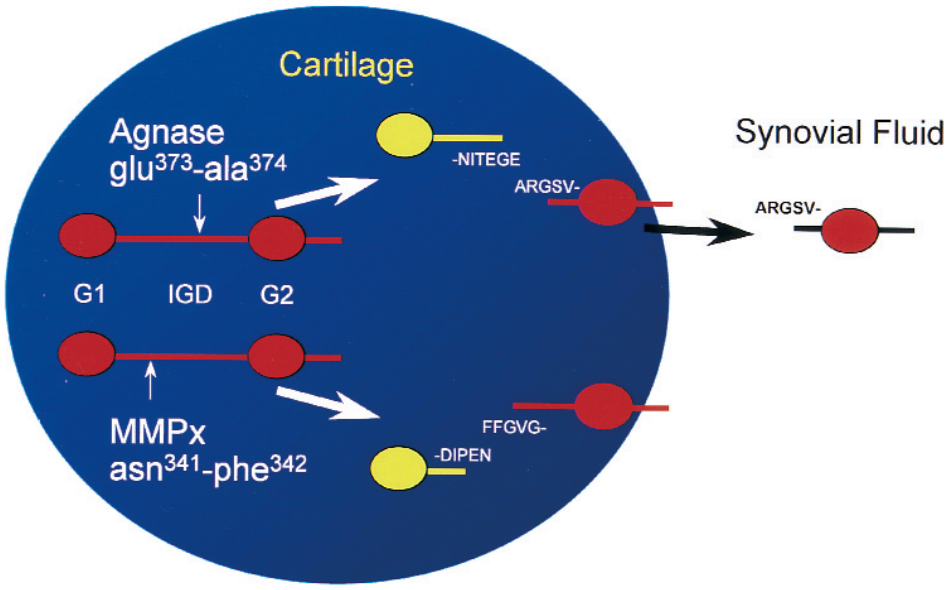

Figure 9. Degradation of aggrecan occurs through at least 2 distinct pathways: Cleavage of the molecule by aggrecanase(s) (Agnase) occurs between glu^{373}-ala^{374} in the interglobular domain (IGD) between the G1 and G2 regions of the core protein, generating the neoepitopes -NITEG and ARGSV-. Cleavage by matrix metalloproteinases (MMP; e.g., stromelysin) occurs between asn^{341}-phe^{342} and generates the neoepitopes -DIPEN and FFGVG-. The products of aggregan cleavage, particularly the C-terminal fragments such as that carrying the neoepitope ARGSV, may appear in the SF. Modified from Lark, *et al*. J Clin Invest 1997;100:93-106.

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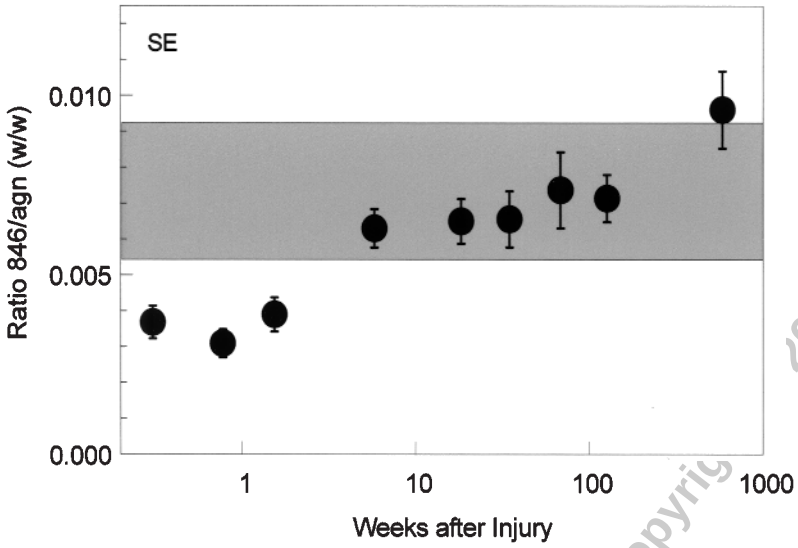

Figure 10. Ratio of the SF level of epitope 846 (a marker of newly synthesized proteoglycans) to the level of all aggrecan (agn) fragments in cross-sectional analyses of patients who suffered a knee injury. Broad horizontal bar depicts the normal range. The bars represent standard error. Note changes in the ratio with time after injury, suggesting dynamic changes in aggrecan synthesis and/or preferential degradation of different tissue pools of aggrecan. Modified from Lohmander, *et al*. Arthritis Rheum 1999;42:534-4.

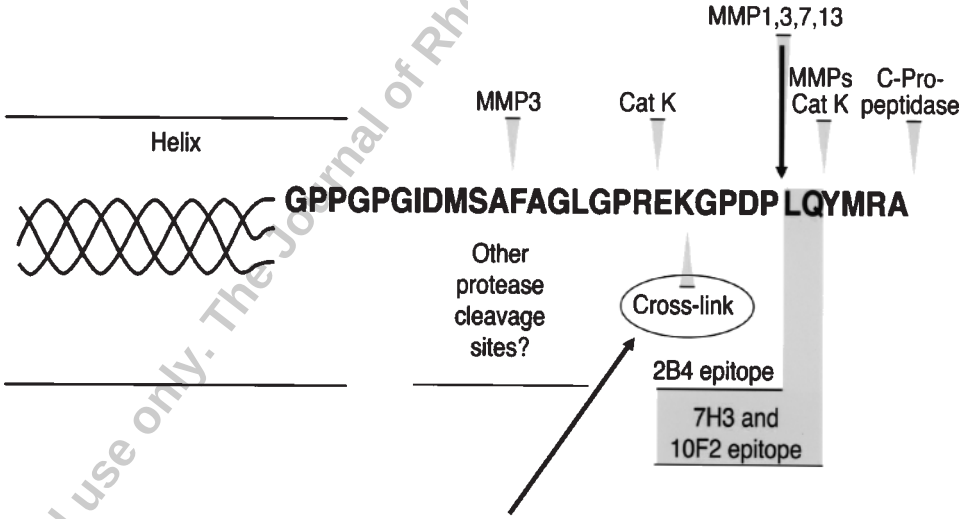

Figure 11. The C-telopeptide domain of one of the alpha-chains of type II collagen. Several different proteases are capable of cleaving different sites in this domain, some of which are depicted. The location of the crosslink is shown. Cleavage of this telopeptide domain generates several neoepitopes that can be detected by monoclonal antibodies. Soluble fragments containing, e.g., the 2B4 EKGPDP-neoepitope and the crosslink, are generated by proteolytic action against this site and one or more sites located in the triple helical domain. Such fragments can be detected by immunoassay in SF, serum/plasma, and urine. Modified from Lohmander, *et al*. Arthritis Rheum 2003;48:3130-9; and Atley, *et al*. Orthop Res Soc 1998;23:850.

of protease activity soon after injury, this could well represent a point of no return and lead to development of OA.

Where, then, do we stand today in our effort to apply biomarkers as surrogate outcome measures? Certainly, we would like to use biomarkers to identify patients who are at risk for rapid progression of OA. Through the use of assays that are much more specific than the ones we have used in the past, e.g., the type II collagen assay developed by Eyre[5],

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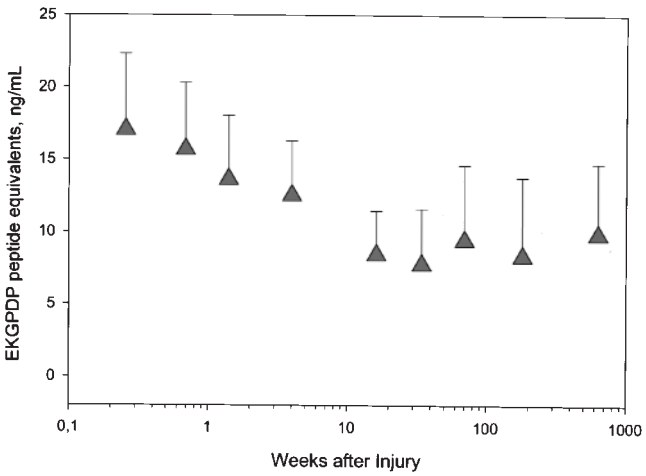

Figure 12. Cross-sectional measurements of collagen II "crosslinked fragments" of the C-telopeptide of type II collagen in knee joint fluid from knees of patients with various intervals after knee injury, as detected by the monoclonal antibody, mAb to the 2B4. Results are expressed as ng of EKGPDP neoepeptide equivalent per ml SF. With permission, from Lohmander, *et al*. Arthritis Rheum 2003;48:3130-9.

we are beginning to see some hints that this may be possible. Assuming that effective DMOAD become available, we may be able to use these assays for this purpose. However, validation of markers as predictors of OA progression is not an "all-or-none" phenomenon; indeed, by the time we have fully validated a particular marker we may not need it any more because we will already have the outcome data for which we needed it originally.

What is the gold standard against which we would attempt to validate a potential marker? Radiographs are not useful for this purpose. The primary measure must be patient-relevant. Clearly, we will always find a limited correlation between structural outcomes such as radiography and patient-relevant outcomes. Nonetheless, this should not deter us from attempting to include biomarker measurements in a risk factor profile. In the future, we may use patient demographics, signs and symptoms, radiographic changes, and perhaps a genetic profile in addition to biomarker data to facilitate the development of risk profiles. With this approach, application of biomarker technology may help increase our ability to diagnose and manage OA.

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